academicJournals

Vol. 10(3), pp. 34-44, March 2018
DOI: 10.5897/JABSD2017.0289
Article Number: CD7DFD156070
ISSN 2141-2340

http://www.academicjournals.org/JABSD

Journal of Agricultural Biotechnology and Sustainable Development

Full length Research Paper

Hydroponic screening and characterization of aluminium tolerance on finger millet (*Eleusine coracana* (L.) Gaertn) accessions

Haftom Brhane[1*], Edossa Fikru[2], Teklehaimanot Haileselassie[3] and Kassahun Tesfaye[3]

[1]Department of Biology, College of Natural and Computational Science, Haramaya University, Ethiopia.
[2]School of Agriculture, Medawelabu University, Ethiopia.
[3]Institite of Biotechnology, AAU, Addis Ababa, Ethiopia.

Received 3 May, 2017; Accepted 10 January, 2018

Biotic and abiotic stress combined with the use of less productive local cultivars cause low production of finger millet in Ethiopia. This research was conducted to investigate acidity tolerance of finger millet accessions. Preliminary screening was done on 288 accessions and six improved national cultivars of finger millet. Twenty randomly selected and surface-sterilized seeds of each germplasm were wrapped and germinated in a tissue paper in Petri dishes. Thirty six hours-old seedlings of uniform size were transferred to the nutrient solutions having 500 µM KNO_3, 500 µM $CaCl_2$, 500 µM NH_4NO_3, 150 µM $MgSO_4.7H_2O$, 10 µM KH_2PO_4, 2 µM $FeCl_3$ (III) and 112.5 µM $Al_2(SO_4)_3.18H_2O$ and allowed to grow for a further 8 days along with tolerant and susceptible references. Characterization with (112.5 µM) and without (0 µM) Al conditions was also done on 80 accessions. After eight days root and shoot length of seedlings were measured using a ruler, while fresh weight of these seedlings was taken using a digital balance. Mean separation and analysis of variance on each treatment was conducted using SPSS software. Relative total root length (RTRL) and root growth inhibition (RGI) were also estimated. From screening of 288 accessions, 75 (26.04%) of them were Al tolerant, while 213 (73.95%) of them were medium to susceptible. From characterization, 63 (78.75%) showed significant Al stress in root length, 23 (28.75%) in fresh weight, while no distinct and visible symptom were observed in shoot growth. The study clearly showed the possibility of developing lines and genotypes that can tolerate acidity in Ethiopian context and support agricultural development in acidic soils in the country and in the world.

Key words: Acid soils, finger millet, hydroponics, screening, plant roots.

INTRODUCTION

Acid soils (with a pH of 5.5 or lower) are among the most important limitations to agricultural production. It has been estimated that 15% of the world's soil is acidic and that over 50% of the world's potentially arable lands are acidic (von Uexküii and Mutert, 1995). Aluminium (Al^{3+}) ranks third in abundance among the earth's crust

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*Corresponding author. E-mail: haftom1@gmail.com.

elements, after oxygen and silicon, and is the most abundant metallic element. A large amount of Al is incorporated into aluminosilicate soil minerals and very small quantities appear in the soluble form, capable of influencing biological systems (Silva et al., 2012). When pH drops below 5.5, aluminosilicate clays and aluminium hydroxide minerals begin to dissolve, releasing aluminium-hydroxyl cations and Al^{3+} then it exchanges with other cations. The chemistry of Al^{3+} in soil solution is complicated by the fact that soluble inorganic (such as sulfate and fluoride) and organic ligands form complexes with Al^{3+}. Whether a ligand increases or decrease aluminium solubility depends on the particular aluminium-ligand complex and its tendency to remain in solution or precipitate. The mononuclear Al^{3+} species is considered as the most toxic form of aluminium (Kochian, 1995). Aluminium bioavailability and, in consequence toxicity, is mainly restricted to acidic environment.

At high concentrations, Al can be a serious threat to agricultural production because it inhibits growth of the roots through various mechanisms, inducing oxidative stress (Zheng and Yang, 2005), callose induction, peroxidation of the cellular membrane, aluminium accumulation and nutrient imbalances and that ends with cell death (May and Nordstrom, 1991). There is considerable variability in A1 tolerance within species and this has been useful to breeders in developing Al-tolerant cultivars of various crops. Generally, there are two main types of Al tolerance mechanisms: (a) those that exclude Al from the root cells and (b) those that allow Al to be tolerated once it has entered the plant cells (Barceló and Poschenrieder, 2002; Kochian et al., 2005).

Eleusine coracana commonly known as finger millet or Ragi is cultivated for its grain in many parts of Africa and India (Hilu and Johason, 1992). Archeological studies confirmed domestication of *E. coracana* started around 5000 B.C. in Western Uganda and highlands of Ethiopia; and it arrived in India much earlier, probably more than 3000 years ago (Hilu et al., 1979). It is a versatile grain that can be used in many different types of food. It is eaten by grinding the grains up for porridge and other food items. Sometimes it is ground into flour and used for bread or various baked products like '*injera'* in Ethiopia. Finger millet is particularly rich in dietary fiber and minerals such as calcium, proteins, phosphorus, amino acids, and iron (Asrat and Frew, 2001) as compared to major cereals grown in Ethiopia. In addition to being nutritious, millets are also considered as healthy food. The grains of most millets do not contain gluten, a substance that causes celiac disease or other forms of allergies. Babu et al. (1987) reported that some high-yielding varieties also contain high protein content (8 to 12%) and also rich in calcium content (294 to 390 mg/100 g). Even though it is an important food security crop, production of the crop is inconsistent due to biotic and abiotic stresses and aluminium toxicity is one of the major factors. Hence, this work was initiated with the aim of screening finger millet accessions and varieties for their Al tolerance in order to enhance the productivity of finger millet in Ethiopia and in the world.

MATERIALS AND METHODS

Equipment setup

Dense narrow holes were introduced into as many Eppendorff tubes as required in such a way that the holes did not allow finger millet seeds to pass through but rather allowed in air bubbles for aerating the seedlings in the tube. "Rack" like plates to hold the perforated Eppendorff were made from jar plastic plate by introducing wide holes capable of holding and submerging Eppendorff tubes in the nutrient solution (Haftom et al., 2017). White plastic dishes were used as solution container with adjustable lids.

Nutrient solution culture

Nutrient solution culture prepared according to Delhaize et al. (2004) and composed of 500 µM KNO_3, 500 µM $CaCl_2$, 500 µM NH_4NO_3, 150 µM $MgSO_4.7H_2O$, 10 µM KH_2PO_4, 2 µM $FeCl_3$ (III) and 112.5 µM $Al_2\ (SO_4)_3.18H_2O$.

Plant and germination conditions

Three hundred accessions and six improved national cultivars of finger millet (*E. coracana*) were obtained from Ethiopian Institute of Biodiversity (EIB) and Nekemte Agricultural Finger Millet Research Center, respectively. Twenty randomly selected and surface-sterilized seeds of each germplasms were wrapped and germinated in a tissue paper, moistened with distilled water, in Petri dishes. Thirty 6 h-old seedlings of uniform size were transferred to the nutrient solutions and allowed to grow for a further 8 days along with tolerant (Gute variety) and susceptible (Necho variety) references.

Treatments

Preliminary screen of 300 accessions was carried on the threshold toxicity level of Al (112.5 µM) on six successive groups. Characterization with (112.5 µM) and without (0 µM) Al conditions was also done on 80 Ethiopian finger millet accessions. The control experiment also included all the above nutrients except $Al_2\ (SO_4)_3.18H_2O$. The experiment was laid down in Randomized Complete Block Design (RCBD) with three replications. The pH of the nutrient was adjusted to 4.3 by using 1M HCl and the solution was renewed for every 24 h.

Data recording and statistical analysis

After eight days, root and shoot length of seedlings were measured using a ruler, while fresh weight of the seedlings was taken using a digital balance (Version No. 339, capacity 210 AE Adam ® with 0.0001 precision). Mean and analysis of variance (ANOVA) on each treatment was conducted using SPSS software version 20. Tukey HSD was used to make pair wise mean comparison of each germplasm under control and Al-treated conditions. Relative total root length (RTRL) and root growth inhibition (RGI) were also estimated using the following method (Mendes et al., 1984).

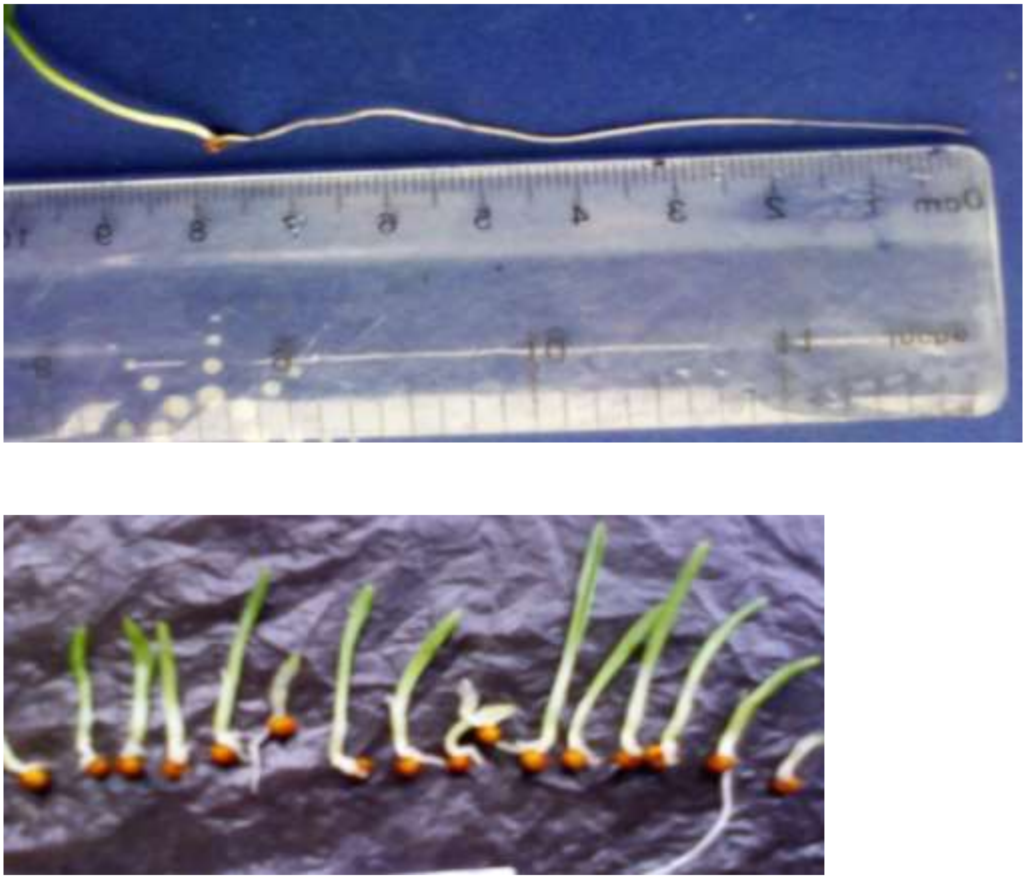

Figure 1. Sample pictures of 8-day old seedlings showing root length difference between different varieties grown at 0 µM (control) above 112.5µM below Al^{3+} concentrations.

$$\text{RTRL} = \frac{\text{Root length undertreatment (Al)}}{\text{Root Length under control (without Al)}} \times 100$$

$$\text{RGI} = 100 - \text{RTRL}$$

where RTRL is relative total root length and RGI is the root growth inhibition.

RESULTS AND DISCUSSION

Screening of finger millet accession for Al^{3+} trait response

A total of 288 accessions were screened in successive six groups, each group contained 50 accessions including the two references. The average root length screened in group one varied from 0.20 to 2.30 cm, while the references varieties Gute and Necho showed 2.11 and 1.54 cm, respectively. Furthermore, accessions screened in group two had an average root length ranging from 0.1 to 2.76 cm, while Gute and Necho had 1.92 and 0.12 cm, respectively. Similarly in group three it ranges from 0.11 to 2.61 cm, while Gute is 2.30 cm and Necho is 0.93 cm. Accessions screened in group four showed better average root growth as compared to the other batches and varied from 0.67 to 0.31 cm, while Gute and Necho produced 1.61 and 0.31 cm, respectively. The root length in accessions of group five was between 0.32 to 3.00 cm, Gute and Necho were 1.94 and 1.16 cm, respectively. Likewise, the performance of accessions of group six ranged between 0.17 to 0.27 cm, while Gute and Necho were 1.95 and 0.48cm, respectively details displayed in (Appendix 1 and Figure 1). From the screening result on 288 accessions along with the standard checks in six batches, only a few of them were Al tolerant 75 (26.04%), while 213 (73.95%) of the total accessions showed medium to susceptible tolerance.

Characterization of Ethiopian finger millet for aluminium tolerance

Out of a total of 80 accessions characterized for further evaluation with and without Al conditions, 74 were

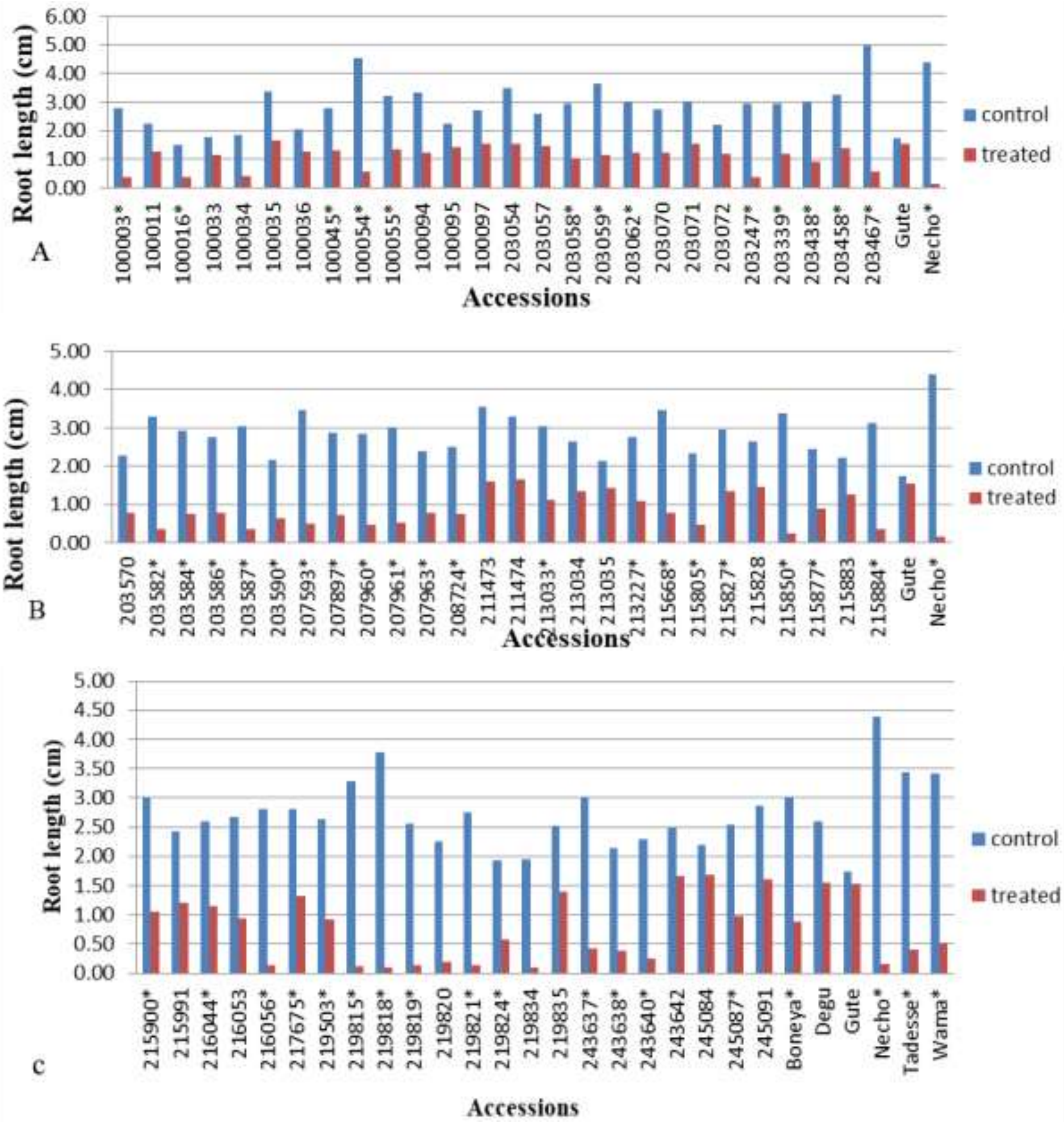

Figure 2. Effect of Al toxicity on root length on 80 finger millet accessions grown under treated; 112.5 µM and control; 0 µM Al^{3+} under hydroponics.

landrace accessions and six improved national varieties. There was significant (P-value of 0.05) Al induced stress among accessions in root and fresh weight measurement (Figures 3 and 4). In root length, 63 accessions (78.75%) showed significant Al induced stress and 23 (28.75%) in fresh weight, while no distinct and visible symptom of aluminium toxicity were observed in the shoot of finger millet genotypes (Figure 2). High root length inhibition was reported in pigeon pea on 20 µM $AlCl_3$ (Choudhary et al., 2011) and in maize at 20 µM (Wagatsuma et al., 2005). The present study also confirmed the inhibition of root growth at 112.5 µM due to aluminium phytotoxicity. Root growth inhibition is considered to be the primary consequence of aluminium toxicity, resulting in a smaller volume of soil explored by the plant roots, consequently reducing its mineral nutrition and water absorption. Furthermore, it reduces cell membrane permeability and binds to the phosphate groups of the deoxyribonucleic acid decreasing replication and transcription activity and also cell division inhibition (Kochian et al., 2005).

In the present study, no distinct and visible symptoms of aluminium toxicity were observed in the shoot growth

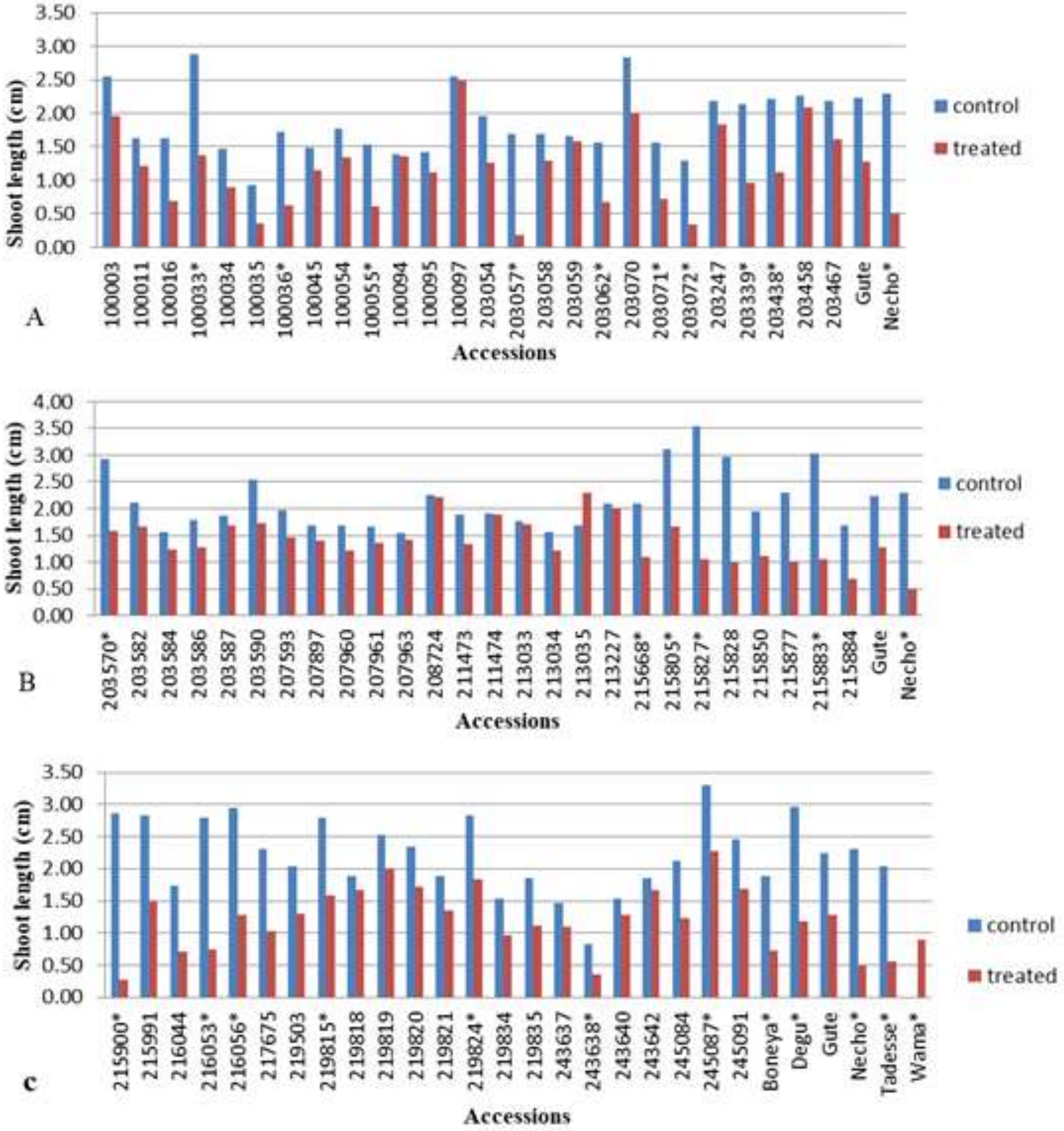

Figure 3. Effect of Al toxicity on shoot length on 80 finger millet accessions grown under treated; 112.5 µM and control; 0 µM Al^{3+} on hydroponics.

of finger millet genotypes, similar with the findings in pigeon pea on 20 µM $AlCl_3$ (Choudhary et al., 2011). Long term exposure might affect nutrient uptake, which can lead to nutritional deficiencies in shoots and leaves (Jiang et al., 2008). The overall effect of aluminium toxicity was expressed on the reduction of yield and its total biomass. Fresh weight reduction in 23 (28.75%) accessions was also observed in this study. The decreased root growth could be the main cause for reduction in fresh weight.

Al tolerance in finger millet genotype as revealed by relative total root length

Accessions collected from Western Ethiopia, Gojam (100033and 213035), Awi (100036 and 243642) and Wellega (100095, 100097, and 245084) were the best seven tolerant accessions, while accessions (219815, 219818, 219819, 219820, and 219821) collected from Northern Ethiopia showed least tolerance levels (Appendix 2). According to Abdenna et al. (2007), acidity

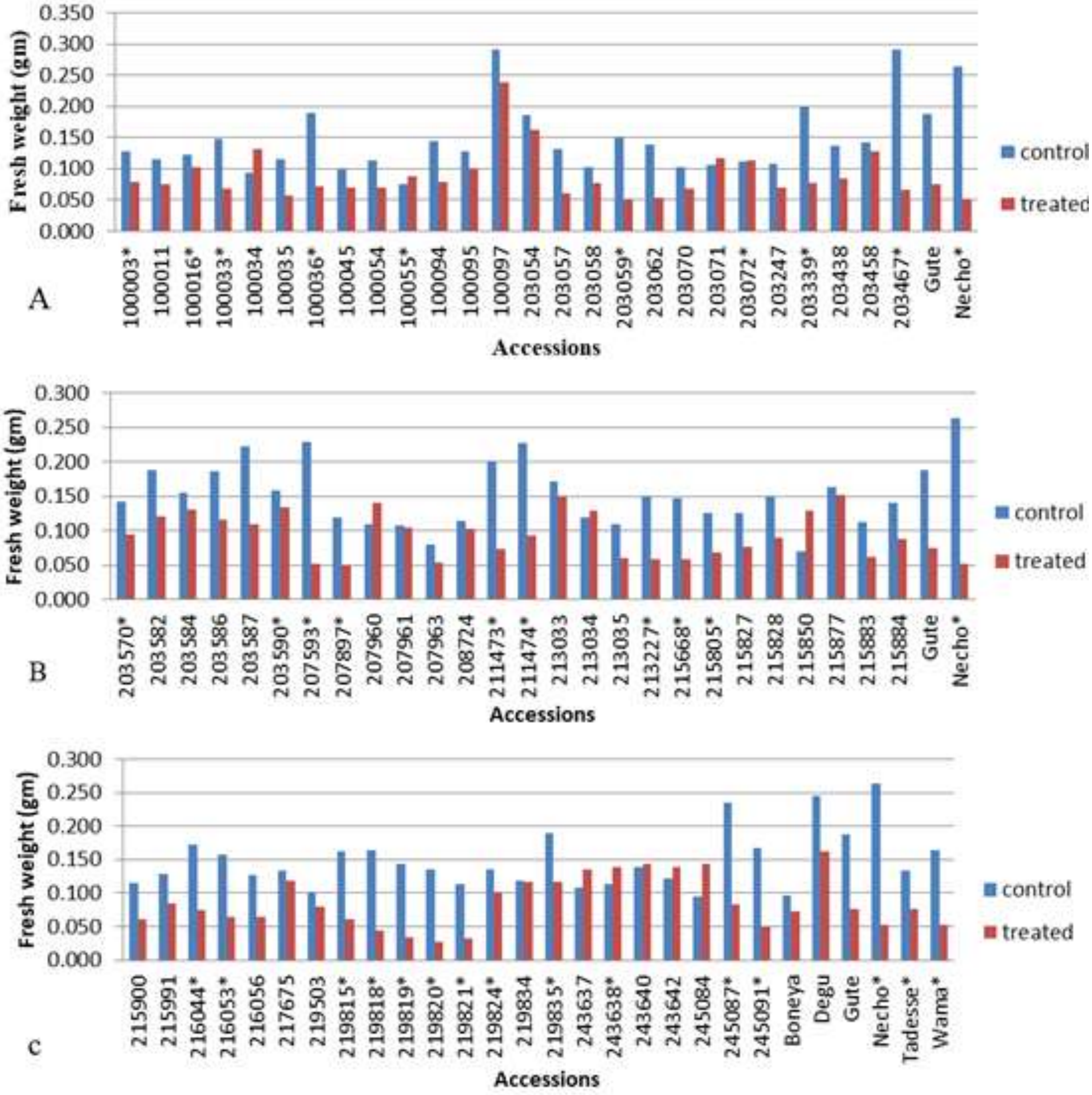

Figure 4. Effect of Al toxicity on fresh weight (g) on 80 finger millet accessions grown under treated; 112.5 µM and control; 0 µM Al^{3+} on hydroponics. Key: (A= 1-26; B = 27-52; C= 53-80). *Significant at p<0.05 Tekuy HSD test.

affected soils are prevalent in the Western and Southern parts of Ethiopia, areas such as Nedjo, Diga, Gimibi and Bedi in Oromiya, Chencha and Sodo in SNNP, and Gozamin and Senan Woreda in Eastern Gojam and Awi zone in West Amhara region. In the Western and Eastern Wellega zones in particular, the large proportion of exchangeable acidity was due to exchangeable aluminium while at West Showa zone, it was due to exchangeable hydrogen. Moreover, accessions collected from these areas were found to be Al^{3+} tolerant, this is mainly due to enhanced tolerance against Al concentration that were developed due to long term exposure to soil acidity in this region. This may also be due to the fact that they exclude Al from the root cells and allow Al to be tolerated once it has entered the plant cells (Barceló and Poschenrieder, 2002; Kochian et al., 2005).

Conclusions

Among national varieties, Necho and Wama as was relatively Al sensitive as revealed by root growth inhibition compared to other varieties of finger millet. Thus, these varieties should not be recommended in areas where soil acidity is predominant. However, Gute and Degu varieties were relatively Al tolerant as revealed by root growth performance and can be promoted in areas where soil acidity is a challenge. Root length (RL) was affected more by Al toxicity than shoot length (SL). The impact of Al toxicity on finger millet germplasm became intense upon toxicity level increments. This study is the first of its kind to evaluate the performance of Ethiopian finger millet to Al-toxicity. The study clearly showed the possibility of developing lines and genotypes that can tolerate acidity in Ethiopian context and support agricultural development in acidic soil areas in the country.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Appendix 1A. Mean root length ± standard error on 300 accessions and two standard checks grown under hydroponics at 112.5 µM Al concentration.

S/N	ACC.	M±SE	S/N	ACC.	M±SE	S/N	ACC.	M±S.E	S/N	ACC.	M±SE
1	Gute	1.111±0.02	41	9355	1.889±0.56	81	100086	0.833±0.35	121	203576	0.511±0.10
2	Necho	0.378±0.15	42	9356	1.455±0.20	82	100088	1.189±0.89	122	203577	0.978±0.35
3	9314	1.611±0.42	43	9357	1.933±0.35	83	100089	2.578±0.20	123	203581	0.789±0.10
4	9315	0.556±0.04	44	9358	2.022±0.55	84	100091	1.992±0.08	124	203582	0.511±0.10
5	9316	0.922±0.52	45	9359	1.700±0.4	85	100092	1.589±0.21	125	203583	0.455±0.187
6	9317	0.933±0.13	46	9360	2.255±0.43	86	100094	0.656±0.43	126	203584	0.833±0.20
7	9318	0.466±0.18	47	9361	1.967±0.49	87	100095	2.344±0.28	127	203586	0.433±0.15
8	9319	1.700±0.30	48	9362	1.111±0.74	88	100096	1.889±0.50	128	203587	0.378±0.12
9	9320	1.611±0.19	49	9363	2.300±0.32	89	100097	2.089±0.26	129	203588	0.778±0.35
10	9321	0.311±0.06	50	9513	2.022±0.02	90	203054	1.733±0.48	130	203589	1.711±0.38
11	9322	1.544±0.16	51	100001	0.167±0.06	91	203055	1.922±0.49	131	203590	0.111±0.1
12	9323	1.400±0.48	52	100002	0.189±0.08	92	203056	1.544±0.38	132	203591	0.255±0.04
13	9324	0.200±0.01	53	100003	0.100±0.00	93	203057	2.333±0.32	133	203592	0.644±0.34
14	9325	0.956±0.39	54	100005	0.100±0.00	94	203058	1.267±0.68	134	203593	0.678±0.23
15	9326	0.611±0.34	55	100006	0.911±0.30	95	203059	1.500±0.40	135	204749	0.933±0.13
16	9327	0.778±0.18	56	100009	0.355±0.137	96	203062	2.189±0.14	136	204750	0.789±0.11
17	9328	1.233±0.58	57	100010	0.133±0.01	97	203069	1.189±0.44	137	204751	0.311±0.09
18	9329	1.989±0.31	58	100011	0.889±0.79	98	203070	0.811±0.39	138	207493	1.022±0.27
19	9330	1.700±0.43	59	100012	0.344±0.14	99	203071	2.100±0.11	139	207755	0.455±0.13
20	9331	0.989±0.48	60	100014	0.500±0.40	100	203072	2.444±0.43	140	207756	0.489±0.10
21	9332	1.033±0.40	61	100016	0.122±0.02	101	203247	2.322±0.12	141	207757	0.589±0.18
22	9333	1.733±0.28	62	100017	0.656±0.28	102	203339	2.067±0.40	142	207897	0.700±0.24
23	9334	0.533±0.33	63	100018	1.889±0.17	103	203348	2.044±0.19	143	207960	0.578±0.12
24	9335	2.056±0.36	64	100019	1.055±0.49	104	203367	1.811±0.13	144	207961	0.989±0.23
25	9336	2.211±0.29	65	100031	0.289±0.09	105	203422	1.733±0.45	145	207963	0.389±0.14
26	9337	1.367±0.69	66	100033	1.478±0.16	106	203438	1.467±0.811	146	208442	0.955±0.28
27	9338	2.067±0.24	67	100034	0.255±0.07	107	203458	2.611±0.27	147	208443	0.755±0.13
28	9339	2.156±0.29	68	100035	2.278±0.54	108	203467	1.533±0.13	148	208445	0.589±0.11
29	9340	1.367±0.37	69	100036	2.033±0.25	109	203477	0.711±0.20	149	208724	1.778±0.27
30	9341	1.522±0.43	70	100045	2.767±0.19	110	203483	1.022±0.18	150	208726	0.789±0.04
31	9342	0.399±0.12	71	100049	1.244±0.39	111	203486	1.422±0.14	151	211029	0.756±0.09
32	9344	1.322±0.48	72	100052	1.456±0.66	112	203491	1.567±0.35	152	211473	0.844±0.13
33	9345	1.255±0.33	73	100054	0.411±0.23	113	203503	1.422±0.02	153	211474	0.755±0.05
34	9347	0.867±0.27	74	100055	2.122±0.54	114	203526	1.133±0.26	154	212461	0.589±0.32
35	9348	1.878±0.29	75	100056	1.289±0.22	115	203531	1.767±0.25	155	212462	0.600±0.50
36	9349	0.956±0.29	76	100061	1.467±0.30	116	203552	0.855±0.21	156	212692	0.178±0.06

Appendix 1A. Contd.

37	9350	0.944±0.47	77	100063	0.855±0.48	117	203570	1.478±0.23	157	212693	0.233±0.06
38	9351	1.789±0.50	78	100064	1.100±0.27	118	203571	0.511±0.04	158	212694	0.444±0.19
39	9352	1.411±0.70	79	100065	0.811±0.36	119	203573	1.300±0.28	159	213032	0.422±0.22
40	9354	2.267±0.21	80	100076	1.989±0.32	120	203575	0.711±0.09	160	213033	0.200±0.03
161	213034	3.133±0.21	201	215958	0.533±0.11	241	223006	0.244±0.14	281	245088	1.067±0.30
162	213035	1.889±0.48	202	215961	0.789±0.06	242	223007	1.167±0.36	282	245091	1.044±0.24
163	213227	1.789±0.06	203	215969	0.778±0.54	243	223008	0.733±0.33			
164	213835	0.411±0.06	204	215975	0.444±0.29	244	223009	0.822±0.24			
165	214207	0.211±0.01	205	215985	0.522±0.37	245	223011	0.533±0.20			
166	214208	0.222±0.04	206	215987	0.322±0.06	246	223013	0.667±0.27			
167	214210	0.789±0.62	207	215991	2.567±0.06	247	223014	0.289±0.07			
168	214987	0.322±0.07	208	216044	2.033±0.60	248	223016	0.322±0.09			
169	214990	1.600±0.55	209	216053	3.000±0.40	249	223017	1.100±0.21			
170	214995	0.656±0.08	210	216054	1.133±0.54	250	223018	0.878±0.48			
171	215668	1.667±0.35	211	216056	2.156±0.40	251	223019	0.522±0.16			
172	215800	0.478±0.31	212	217674	1.467±0.69	252	223024	0.222±0.06			
173	215801	0.656±0.19	213	217675	2.211±0.05	253	223025	1.144±0.04			
174	215802	1.833±0.59	214	217677	2.711±0.35	254	223026	0.167±0.01			
175	215803	1.500±0.86	215	219814	0.944±0.21	255	223027	0.222±0.12			
176	215804	1.455±0.17	216	219815	0.678±0.12	256	223028	0.322±0.20			
177	215805	1.678±0.72	217	219818	0.633±0.18	257	223029	1.478±0.52			
178	215826	0.689±0.47	218	219819	0.700±0.29	258	223031	1.978±0.09			
179	215827	1.067±0.67	219	219820	1.889±0.27	259	223033	0.289±0.13			
180	215828	1.500±0.86	220	219821	1.244±0.08	260	223034	0.633±0.20			
181	215829	0.211±0.04	221	219824	0.733±0.08	261	223037	0.911±0.51			
182	215830	1.778±0.89	222	219834	1.278±0.31	262	223038	0.344±0.12			
183	215844	2.400±0.11	223	219835	1.478±0.26	263	223039	0.200±0.01			
184	215850	1.722±0.32	224	219838	1.678±0.14	264	223144	1.556±0.39			
185	215855	0.744±0.51	225	220090	1.400±0.15	265	243634	1.789±0.16			
186	215863	2.000±0.16	226	221697	1.044±0.37	266	243635	0.422±0.17			
187	215865	1.622±0.42	227	221699	1.678±0.21	267	243636	2.133±0.06			
188	215874	0.878±0.51	228	222975	0.389±0.06	268	243637	1.211±0.68			
189	215877	2.078±0.46	229	222978	0.422±0.13	269	243638	2.700±0.35			
190	215878	0.3±0.10	230	222980	1.167±0.27	270	243639	1.122±0.42			
191	215882	0.411±0.11	231	222990	0.833±0.20	271	243640	1.711±0.57			
192	215883	2.111±0.29	232	222991	1.455±0.29	272	243641	1.599±0.38			

Appendix 1A. Contd.

193	215884	1.811±0.11	233	222992	1.344±0.29	273	243642	2.022±0.09
194	215891	1.455±0.12	234	222994	0.844±0.14	274	243643	1.989±0.02
195	215900	2.467±0.30	235	222999	1.378±0.16	275	243644	1.556±0.19
196	215907	1.511±0.67	236	223001	0.589±0.07	276	244798	1.167±0.46
197	215909	1.167±0.03	237	223002	1.689±0.27	277	245084	2.056±0.12
198	215912	1.567±0.33	238	223003	0.844±0.22	278	245085	0.633±0.28
199	215917	0.822±0.56	239	223004	0.755±0.48	279	245086	1.833±0.15
200	215921	0.367±0.18	240	223005	1.956±0.29	280	245087	1.211±0.23

Appendix 2. Mean root length ± standard error on 300 accessions and two standard checks grown under hydroponics at 112.5 µM Al concentration.

Acc.	0 µM	112.5 µM	RTRL	Acc.	0 µM	112.5 µM	RTRL	Acc.	0 µM	112.5 µM	RTRL
100003	2.978	0.357	0.12	203582	3.3	0.369	0.112	216044	2.6	1.144	0.44
100011	2.256	1.289	0.571	203584	2.933	0.744	0.254	216053	2.667	0.944	0.354
100016	1.522	0.389	0.255	203586	2.767	0.767	0.277	216056	2.8	0.133	0.048
100033	1.789	1.144	0.64	203587	3.044	0.367	0.12	217675	2.8	1.311	0.468
100034	2.867	0.411	0.143	203590	2.156	0.644	0.299	219503	2.644	0.922	0.349
100035	3.078	1.656	0.538	207593	3.456	0.511	0.148	219815	3.289	0.111	0.034
100036	2.067	1.267	0.613	207897	2.867	0.711	0.248	219818	3.978	0.104	0.026
100045	2.8	1.311	0.468	207960	2.833	0.468	0.165	219819	3.567	0.133	0.037
100054	4.556	0.578	0.127	207961	3.011	0.522	0.173	219820	3.556	0.189	0.053
100055	3.2	1.333	0.417	207963	2.389	0.767	0.321	219821	2.744	0.133	0.049
100094	3.333	1.222	0.367	208724	2.511	0.756	0.301	219824	3.933	0.567	0.144
100095	2.233	1.422	0.636	211473	3.556	1.6	0.45	219834	1.944	0.089	0.046
100097	2.222	1.544	0.693	211474	3.289	1.656	0.503	219835	2.522	1.389	0.551
203054	3.511	1.556	0.443	213033	3.044	1.133	0.372	243637	3.022	0.422	0.14
203057	2.611	1.467	0.562	213034	2.656	1.344	0.506	243638	2.333	0.389	0.167
203058	2.933	1.044	0.356	213035	2.133	1.422	0.667	243640	2.3	0.244	0.106
203059	3.633	1.144	0.315	213227	2.744	1.1	0.401	243642	2.5	1.667	0.667
203062	2.989	1.233	0.413	215668	3.456	0.767	0.222	245084	2.189	1.689	0.772
203070	2.744	1.222	0.445	215805	2.333	0.467	0.2	245087	2.533	0.978	0.386
203071	3.022	1.556	0.515	215827	2.967	1.344	0.453	245091	2.867	1.611	0.562
203072	2.222	1.2	0.54	215828	2.656	1.467	0.552	Boneya	3.011	0.878	0.292
203247	2.933	0.378	0.129	215850	3.378	0.256	0.076	Degu	2.6	1.54	0.592
203339	2.933	1.178	0.402	215877	2.444	0.889	0.364	Gute	2.744	1.533	0.559

Appendix 2. Contd.

203438	2.967	0.9	0.303	215883	2.233	1.267	0.567	Necho	4.389	0.156	0.035
203458	3.267	1.378	0.422	215884	3.122	0.344	0.11	Tadesse	3.444	0.4	0.116
203467	5.022	0.556	0.111	215900	2.989	1.056	0.353	Wama	3.422	0.522	0.153
203570	2.289	0.789	0.345	215991	2.422	1.2	0.495	-	-	-	-